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Molecular characterization of human enteric viruses in food, water samples, and surface swabs in Sicily

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ABSTRACT

Objectives: Enteric viruses are responsible for foodborne and waterborne infections affecting a large number of people. Data on food and water viral contamination in the south of Italy (Sicily) are scarce and fragmentary. The aim of this study was to evaluate the presence of viral contamination in food, water samples, and surface swabs collected in Sicily

Methods: The survey was conducted on 108 shellfish, 23 water samples (seawater, pipe water, and torrent water), 52 vegetables, one peach and 17 berries, 11 gastronomic preparations containing fish products and/or raw vegetables, and 28 surface swabs. Hepatitis A virus (HAV), genogroup GI, GII, and GIV norovirus (NoV), enterovirus (EV), rotavirus (RoV), hepatitis E virus (HEV), adenovirus (AdV), and bocavirus (BoV) were detected by nested (RT) PCR, real-time PCR, and sequence analysis.

Results: The most frequently detected viruses in shellfish were HAV (13%), NoV (18.5%), and EV (7.4%). Bocavirus was found in 3.7%, HEV in 0.9%, and AdV in 1.9% of the molluscs. Of the 23 water samples, 21.7% were positive for GII NoV and 4.3% for RoV and HEV genotype 3. Of the 70 vegetable samples, 2.9% were positive for NoV GI (GI.5 and GI.6), 2.9% for EV, and 1.4% for HEV. In the gastronomic preparations, only one EV (9%) was detected. No enteric viruses were detected in the berries, fruit, or swabs analyzed.

Conclusions: Molecular surveillance of water and food samples clearly demonstrated that human pathogenic viruses are widely found in aquatic environments and on vegetables, and confirmed the role of vegetables and bivalve molluscs as the main reservoirs.

Introduction

Numerous viruses of human or animal origin are found in the environment and infect people via water and food: bivalve molluscs, vegetables, and prepared foods are classified by the World Health Organization (WHO) as priority hazards. According to a report by the European Food Safety Authority (EFSA) (EFSA, 2016), viruses were, for the first time in 2014, the most commonly detected (20.4%) causative agent in foodborne outbreaks. Environmentally transmitted viruses include major etiological agents of gastroenteritis, meningitis, and hepatitis. Most of these viruses belong to the families *Adenoviridae*,*Caliciviridae*, *Hepeviridae*, *Picornaviridae*, and *Reoviridae* (Dubois et al., 1997; Muscillo et al., 2001; Lodder and de Roda Husman, 2005). These pathogens are excreted in large quantities in the faeces of infected individuals (up to 10^{11} viral particles per gram of stool) (Fong and Lipp, 2005) and are routinely introduced into the environment through the discharge of treated and untreated waste that can be transported through groundwater, estuarine water, seawater, and rivers (La Rosa and Muscillo, 2012; La Rosa et al., 2012; Okoh et al., 2010).

The consumption of fish products, particularly edible lamellibranch molluscs (ELM), is a risk for human health because of their capacity to filter, accumulate, and concentrate pathogens present in the water. Vegetables and berries, if irrigated with contaminated water, can retain microbial agents on their surfaces, including enteric viruses (Petrinca et al., 2009; La Rosa et al., 2010a; Severi et al., 2015; Scavia et al., 2017; Iaconelli et al., 2017), and therefore

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play an important role in the determination of environmental and food contamination.

Hepatitis A virus (HAV), hepatitis E virus (HEV), adenovirus (AdV), norovirus (NoV), and multiple strains of enterovirus (EV) (echoviruses and coxsackievirus) are enteric viruses associated with human wastewater (Lodder and de Roda Husman, 2005). HAV, NoV, and rotavirus (RoV) have been found in shellfish, especially oysters (Bellou et al., 2013), and EV contaminate and survive in brackish and probably saltwater and in shellfish (Patel et al., 2009).

HAV is highly resistant in the environment and is typically transmitted through contaminated foods (raw shellfish, strawberries, etc.) or water (Pinto and Bosch, 2013). Outbreaks were described in 1996–1997 and 2004 in regions of southern Italy (Apulia and Campania) due to the consumption of raw shellfish (Malfait et al., 1996); in 2013 an outbreak was linked to the consumption of frozen mixed berries (Rizzo et al., 2013). Two phylogenetically related but distinct HAV genotype 1A strains were responsible for a small cluster in 2014 and an outbreak in 2015 in Naples, caused by contaminated shellfish (unpublished data).

NoV are a major cause of acute gastroenteritis in children and adults worldwide (Patel et al., 2008; Matthews et al., 2012). NoV can be classified into seven genogroups, GI to GVII (Vinjé, 2015; Zheng et al., 2006). More than 30 genotypes within genogroups GI, GII, and GIV may infect humans (Kroneman et al., 2013), but a single genotype, GII.4, has been associated with the vast majority of NoV-related outbreaks and sporadic cases of acute gastroenteritis worldwide (Bok et al., 2009). Since 1996, there have been at least six human NoV epidemic strains. Several routes of NoV transmission have been identified in many well-documented outbreaks. In general, person-to-person spread is the primary mode of transmission. Foodborne transmission can also play an important role, including infected food handlers. Several waterborne outbreaks have been described, and there is indirect evidence of potential airborne transmission (such as through explosive vomiting occurring during the disease) (La Rosa et al., 2012; Giammanco et al., 2014; Giammanco et al., 2018).

Four species of EV have been classified (A–D): human EV (hEV) type A includes some coxsackievirus A strains; hEV B includes coxsackievirus A9, coxsackievirus B1–6, and most of the echoviruses; and hEV C includes poliovirus 1–3 and some coxsackievirus A strains. The more recently identified hEV have been given individual numbers, from EV68, and are classified amongst all four genotypes (Stanway et al., 2005).

RoV infections are found worldwide and most children are infected during the first 6–9 months of life; it is estimated to cause more than 200 000 deaths annually (Soriano-Gabarró et al., 2006).

Four HEV genotypes are known to infect humans: genotype 1 (G1) and 2 (G2) viruses have been identified exclusively in humans, whereas genotype 3 (G3) and 4 (G4) viruses have been isolated from both humans and animals, mainly pigs and wild boar (Meng, 2009; Pavio et al., 2010). The WHO estimates that 20 million HEV infections occur every year, with over three million acute cases and 57 000 hepatitis E-related deaths (http://www.who.int/mediacentre/factsheets/fs280/en/).

Human AdV (HAdV) are the only human enteric viruses to contain DNA. They are slow growing and are often detected in different environments with other hEV and/or HAV (Puig et al., 1994; Pina et al., 1998). Symptoms of AdV infection include gastroenteritis, pharyngitis, pneumonia, conjunctivitis, and meningoencephalitis (Lenaerts et al., 2008). HAdV have been detected widely in wastewater (both influent and effluent sewage), in surface water, and in recreational water (marine and freshwater samples), as well as in treated and disinfected drinking water (Mena and Gerba, 2009). HAdV have been proposed as indicators for the monitoring of human faecal contamination of water and the efficacy of water purification (La Rosa et al., 2010a,b).

Human BoV (HBoV) is found worldwide in respiratory samples, mainly from children with acute respiratory infections, and in stool samples from patients with gastroenteritis. Four HBoV species are currently included in the Bocavirus genus: HBoV-1, HBoV-2, HBoV-3, and HBoV-4 (Arthur et al., 2009). HBoV has been suggested to cause human disease (Wong-Chew et al., 2017). Water sources are contaminated with HBoV from humans excreting HBoV (Iaconelli et al., 2016; La Rosa et al., 2018).

The objective of this study was to assess the presence of HAV, NoV (GI, GII, and GIV), EV, RoV, HEV, AdV, and BoV in bivalve mollusc shellfish, environmental water, food preparations containing fish products and/or raw vegetables (sushi, sashimi, etc.), vegetables, fruit, berries, and surface swabs in food preparation areas by end-point and real-time PCR/RT-PCR and sequence analysis.

Materials and methods

A total of 108 fresh and frozen shellfish, 70 vegetables, 23 environmental water samples, 11 samples of gastronomic preparations, and 28 surface swabs were collected between 2012 and 2017. All of the samples were screened for HAV, HEV, NoV (GI, GII, and GIV), EV, RoV, AdV, and BoV using molecular detection methods.

Sampling

Shellfish samples were collected from three harvesting areas in the province of Syracuse and five centres in Messina, and from restaurants, fish markets, and shellfish markets, as part of official control monitoring programmes in Sicily (Figure 1). They consisted of 89 *Mytilus galloprovincialis*, one *Mytilus edulis*, 12 *Tapes semidecussatus*, two *Tapes decussatus*, one *Ensis directus*, two *Crassostrea gigas*, and one *Venus verrucosa*.

Water samples included 10 seawater samples, 11 pipe water samples, one from a desalter, and one torrent water sample. They included eight brackish water samples from the mussel farming centre in Syracuse; three pipe water samples from Santo Stefano di Quisquina (AG), where a NoV outbreak occurred in 2011 (Giammanco et al., 2014); three water samples from Lampedusa island (AG), including one from a desalter and two seawater samples where a HAV outbreak occurred in 2014; one torrent water sample from the province of Caltanissetta; eight pipe water samples from the province of Catania where a NoV outbreak occurred in May 2016 (Giammanco et al., 2018) (Figure 1).

Vegetable samples consisted of 51 first range raw vegetables and one fourth range ready-to-eat vegetable (belonging to different brands and from supermarkets, local markets, and farms), one peach, and 17 frozen berries, including two blackberries and 15 mixed berries (composed of blackberries, blueberries, and raspberries). The vegetables included 19 mixed salads, four Swiss chard (*Beta vulgaris* var. *cicla*), two chicory (*Cichorium intybus*), one parsley (*Petroselinum crispum*), five celery (*Apium graveolens*), two escaroles (*Cichorium endivia*), one endive (*Cichorium endivia crispum*), nine local lettuces, three iceberg (*Lactuca sativa*), five romaine lettuce (*Lactuca sativa L.* var. *longifolia*), and one trocadero lettuce (*Lactuca scariola sativa*) (Figure 1).

Food samples included two sea urchin eggs (huevas de erizo, *Paracentrotus lividus*) from Morocco and nine raw vegetable, fish, mollusc, and crustacean based food preparations from restaurants in Palermo.

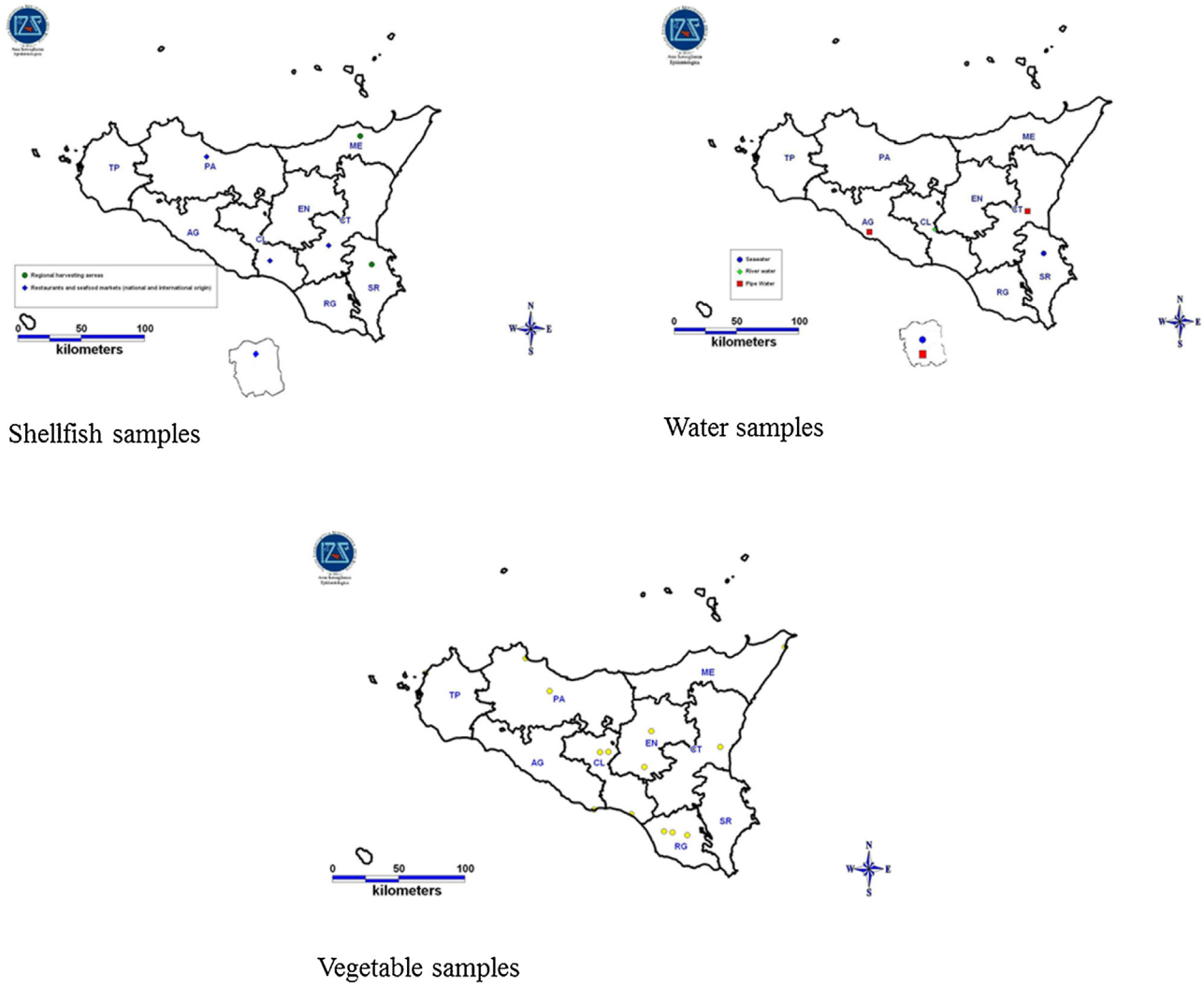

Figure 1. Maps of Sicily showing the locations where the samples were collected.

Finally, 28 swab samples were collected, including 19 surface swabs from two canteens and two restaurants and nine swabs from the hands of workers in restaurants in Palermo.

Preparation of molluscs, foods, gastronomic preparations, and swabs

Each batch of mollusc sample (consisting of 25 g of hepatopancreas), foods, and gastronomic preparations were homogenized with 0.05 M glycine buffer (pH 9.2), processed with a double 1.5 M NaCl PEG 8000 (final concentration of 12.5%) precipitation, followed by high-speed centrifugation (10 000 × *g* for 20 min at +4 °C). The final pellet was resuspended in 3 ml of antibiotic phosphate buffered saline (PBS) and stored at +4 °C overnight (Croci et al., 1999). A parallel extraction from 2 g of hepatopancreas was performed following the ISO technical specification (ISO 15216-2:2013), using treatment with a proteinase K solution. The samples were spiked with 10 µl of titrated mengovirus process control strain MC_0 ($1.6 \times 10^4 TCID_{50}$/ml), to monitor extraction efficiency following the ISO 15216 guidelines. They were digested with 2 ml of proteinase K (0.1 mg/ml) at 37 °C with shaking for 60 min, and then maintained at 60 °C for 15 min to produce inactivation of the enzyme. Finally, the samples were centrifuged at 3000 × *g* for 5 min at room temperature and the supernatants were collected and retained for genome extraction. The swabs were squeezed and centrifuged at 3000 rpm (+4 °C).

Preparation of water samples

Water samples (10 l each) were concentrated through a tangential ultrafiltration system (Sartoflow Slice 200 Benchtop Crossflow System; Sartorius AG, Goettingen, Germany), using appropriate membranes (SG Hydrosart 10 kDa) pre-treated with 300 ml of 3% beef extract (BE) pH 7, by recirculating it for 10 min in the system, at a pressure not higher than 1.30 bar. The elution and recovery of viruses attached to the membranes was performed by washing with 200 ml of 3% BE pH 9.5, to reduce the initial volume to 10–12 ml. The pH of the eluate was then checked with litmus paper and if necessary brought to pH 7 with HCl (Aulicino et al., 1993; Giammanco et al., 2014). The use of ultrafiltration systems as a concentration method to recover viruses from environmental water samples combined with RT-PCR to detect viral genomes has been demonstrated to provide a recovery rate of >50% of multiple viruses (Olszewski et al., 2005) and is at present one of the most sensitive approaches for viral detection.

Preparation of vegetables and berries

Viruses were concentrated from vegetables as follows: 50 g of each sample was coarsely chopped, spiked with 10 μl of titrated mengovirus process control strain to monitor extraction efficiency following the ISO 15216 guidelines, and homogenized with 80 ml of elution buffer (Tris/glycine/BE (TGBE) buffer pH 9.5) (and pectinase if the sample consisted of berries) using a mixer at maximum speed for 1 min. This homogenate was agitated for 20 min at room temperature. Next, the homogenate was centrifuged at 10 000 × *g* for 30 min at 4 °C; the aqueous phase was recovered and its pH was adjusted to 7.2 under constant agitation. PEG 8000 (Sigma Chemical Co., St. Louis, MO, USA)/NaCl was added at a ratio of 1:4 of the volume, and the solution was homogenized by shaking for 1 min and incubated with constant rocking for 60 min (or overnight) at 4 °C. Viruses were concentrated by centrifugation at 10 000 × *g* for 30 min at 4 °C. The supernatant was decanted and discarded and the pellet was compacted by centrifugation at 10 000 × *g* for 5 min at 4 °C. The supernatant was discarded and the pellet was resuspended in 2 ml of PBS with antibiotics and antimycotic (1000 U/ml penicillin G sodium salt, 1 mg/ml streptomycin sulphate, 2.5 μg/ml amphotericin B) and centrifuged at 10 000 × *g* for 15 min. The supernatant was decontaminated by incubating it for 2 h at 4 °C (or overnight). The upper aqueous phase containing viruses was recovered and used for analysis or kept frozen at −20 °C until use.

Nucleic acid extraction

For the extraction of the viral genomes (RNA and DNA), a commercial kit based on the selective binding of nucleic acids to silica magnetic beads was used (NucliSENS miniMAG extraction; bioMérieux Italia S.p.A., Rome, Italy), as described by the manufacturer. In order to compare the extraction results, spin columns were also used for some samples, employing the QIAamp Viral RNA Mini Kit (Qiagen, RNA), by varying the initial volume of the sample to be extracted (560 μl) and the volume of the eluate (100 μl).

Table 1
Molecular methods, primers, and probes used in this study.

Molecular Methods	Primers Probes	Sequence 5'–3'	Target	PCR Product (bp)	Reference
Semi-NestedRT-PCR HAV	AV1 (Rev) AV2 (Fw) AV3 (Rev):	5'-GGAAATGTCTCAGGTACTTTCTTTG-3' 5'-GTTTTGCTCCTCTTTATCATGCTATG-3' 5'-TCCTCAATTGTTGTGATAGC-3'	VP1	First 247 bp Semi-Nested 210 bp	Le Guyader et al., 1994
Real-time RT-PCR HAV	HAV68 (Fw) HAV240 (Rev) HAV150p (Probe)	5'- TCACCGCCGTTTGCCTAG -3' 5'- GGAGAGCCCTGGAAGAAAG -3' FAM 5'-CCTGAACCTGCAGGAATTAA-3' MGB	5'-NCR	173 bp	Costafreda et al., 2006 ISO/TS 15216-2:2013
First RT-PCR GI-GII NoV *Semi-NestedRT-PCR GI NoV* *Semi-NestedRT-PCR GII NoV*	JV12Y 1421-f (Fw) JV13I 1422-r (Rev) GI[c] 1423-f (Fw) JV13I 1422-r (Rev) JV12Y 1421-f (Fw) NoroII-R[c] 1424-r (Rev)	5'-ATACCACTATGATGCAGAYTA-3' 5'-TCATCATCACCATAGAAIGAG-3' 5'-TCNGAAATGGATGTTGG-3' 5'-TCATCATCACCATAGAAIGAG-3' 5'-ATACCACTATGATGCAGAYTA-3' 5'-AGCCAGTGGGCGATGGAATTC-3'	GI-GII NoV RNA polymerase GI NoV RNA polymerase GII NoV RNA polymerase	First 327 bp Semi-Nested 188 bp Semi-Nested 237 bp	Vinje & Koopmans, 1996 Green et al., 1998 Vennema et al., 2002 Boxman et al., 2006
Real-time RT-PCR GI NoV *Real-time RT-PCR GII NoV*	QNIF4 (Fw) NF1LCR (Rev) NVGG1p (Probe) QNIF2 (Fw) COG2R (Rev) QNIFs (Probe)	5'-CGCTGGATGCGNTTCCAT-3' 5'-CCTTAGACGCCATCATCATTTAC-3' FAM 5'-TGGACAGGAGAYCGCRATCT-3' TAMRA 5'-ATGTTCAGRTGGATGAGRTTCTCWGA-3' 5'-TCGACGCCATCTTCATTCACA-3' FAM 5'-AGCACGTGGGAGGGCGATCG-3' TAMRA	ORF2 ORF2	86 bp 89 bp	da Silva et al., 2007 ISO/TS 15216-2:2013
NestedRT-PCR GIV NoV	1531-f (Fw) 1532-r (Rev) 1698-f (Fw) 1699-r (Rev)	5'-GCACTCGGCATCATGACAAAATTCA-3' 5'-GTTTGGGTCCCAATTCCAA-3' 5'-GTACTGGACCAAGGGCCCGA-3' 5'-GAGGTTGCCCGCACCATCCG-3'	ORF1/ORF2	First 995 bp Nested 323 bp	Muscillo et al., 2013
NestedRT-PCR EV	Ent 1-1246 (Fw) Ent 2-1247 (Rev) neEnt 1-1248 (Fw) neEnt 2-1249 (Rev)	5'-CGGTACCTTTGTACGCCTGT-3' 5'-ATTGTCACCATAAGCAGCCA-3' 5'-TCCGGCCCCTGAATGCGGCTA-3' 5'-GAAACACGGACACCCAAAGTA-3'	5'NTR 5'NTR	First 540 bp Nested 123 bp	Pina et al., 1998
NestedRT-PCR HEV	ORF1F-1679-f (Fw) ORF1R-1680-r (Rev) ORF1FN-1681-f (Fw) ORF1RN-1682-r (Rev)	5'-CCAYCAGTTYATHAAGGCTCC-3' 5'-TACCAVCGCTGRACRTC-3' 5'-CTCCTGGCRTYACWACTGC-3' 5'-GGRTGRTTCCAIARVACYTC-3'	ORF1	First 348 bp Nested172 bp	Fogeda et al., 2009
NestedPCR BoV	ID_2028 (Fw) ID_2029 (Rev) ID_2030 (Fw) I1_2030 (Rev)	5'-GAAATGCTTTCTGCTGYTGAAA-3' 5'-GTGGATATACCCACAYCAGAA-3' 5'-GGTGGGTGCTTCCTGGTTA-3' 5'-TCTTGRATTTCATTTTCAGACAT-3'	VP1/VP2	First 543 bp Nested 382 bp	La Rosa et al., 2015 Iaconelli et al., 2016
NestedPCR AdV	ADE1–hexAA1885 (Fw)	5'-GCCGCAGTGGTCTTACATGCACATC-3'	hexon genes	First 301 bp	Formiga-Cruz et al., 2005

Molecular detection and quantification

HAV, NoV (GI/GII/GIV), EV, RoV, HEV, AdV, and BoV were analyzed by molecular methods: nested (RT) PCR and sequencing, and real-time (RT) PCR. The molecular methods, primers, and probes used in this study are shown in Table 1, along with target regions, PCR amplicons, and references.

A semi-nested RT-PCR for HAV was performed using a GeneAmp RNA PCR Core Kit (Applied Biosystems). Semi-nested RT-PCR for GI and GII NoV and a nested RT-PCR for GIV NoV, EV, BoV, and HEV were performed by MyTaq One-Step RT-PCR Kit and MyTaq Red Mix Kit (Bioline). AdV nested PCR was performed using the Taq PCR Core Kit (Qiagen).

The real-time RT-PCR for HAV and GI and GII NoV were prepared using the UltraSense One-Step (Quantitative) qRT-PCR System (Invitrogen) RNA kit, as described in ISO 15216-2:2013. The genome of RoV was amplified by real-time RT-PCR using the TaqMan Universal PCR Master Mix (Applied Biosystems).

Confidence intervals (95%) of the positive results were calculated for proportions.

The positive RT-PCR/PCR products obtained were purified using illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) and were subjected to direct automated sequencing on both strands (BMR Genomics, Padova, Italy). The raw forward and reverse ABI files obtained by sequencing were aligned and assembled into a consensus sequence using MEGA 7 software, and sequences were submitted to BLAST analysis for genotyping at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Results

The results of the two nucleic acid extraction methods (method of magnetic extraction and method employing spin columns) were comparable, and the results obtained from virological analysis are summarized in Table 2.

Of the 108 mollusc samples, 35 (32.4%, 95% CI 24.3–41.7%) were positive for enteric viruses, showing a high positivity for HAV (13%), NoV (18.5%; 10.2% GI (one GI.1, seven GI.2, two GI.3, one GI.8), 5.6% GII, and 2.8% GIV), and EV (7.4%). BoV was found in 3.7%, HEV in 0.9%, and Adv in 1.9% of the molluscs. No RoV was found in shellfish.

The 14 HAV-positive shellfish (one HAV 1A and 13 HAV 1B) were only detected by semi-nested RT-PCR. All 10 GI NoV-positive shellfish were detected by semi-nested RT-PCR and only two of 10 GI NoV by real-time RT-PCR. All six GII NoV-positive shellfish were detected by real-time RT-PCR GII NoV and three of six by semi-nested RT-PCR. The recovery efficiency, determined on the process control mengovirus, was >1% for all samples; thus the results are considered valid according to ISO 15216-2:2013. Of the positive shellfish, 16 were positive by both extraction methods used, while the remaining were positive by either one or the other (13 with proteinase K and 10 with glycine), confirming the results obtained in previous investigations (La Rosa et al., 2012; Mohan et al., 2014), and thus indicating that the positives would have been underestimated if a single method had been used. A Chi-square test was used to examine their association. A p-value of <0.001 was deemed significant.

Of the 23 water samples, six (26%, 95% CI 12.6–46.5%) were positive for enteric viruses. Five water samples (21.7%) were positive for GII NoV (GII.2 and GII.4), only by real-time RT-PCR GII NoV. The superficial freshwater sample was found positive for GII.4 and also for RoV. Only one water sample (4.3%) was positive for HEV (G3). None of the other tested enteric viruses were detected in the water samples.

Of the 70 vegetable samples, five (7.1%, 95% CI 3.1–15.7%) were positive for enteric viruses. Two samples (2.9%) were positive for GI NoV (GI.5 and GI.6), only by semi-nested RT-PCR. EV were detected in two samples (2.9%) and HEV in one sample (1.4%). The recovery efficiency, determined on the process control mengovirus, was >1% for all the samples and thus the results were considered valid according to ISO 15216-2:2013.

In the gastronomic preparations, only one EV (9%) was detected. No enteric viruses were detected in the berries, fruit, or swabs analyzed.

Eleven samples (4.6%) were positive for two or three viruses: 10 ELM and one torrent water sample.

Discussion

Viruses are recognized as a cause of foodborne and waterborne disease transmitted by faecal–oral cycle. Among the main foods involved in the transmission of human enteric viruses are molluscs and fruits and vegetables irrigated with wastewater and/or washed with non-potable water or contaminated by contact with surfaces or the hands of infected personnel during preparation. Also, viral waterborne disease outbreaks associated with contaminated drinking water or recreational water are reported worldwide.

The last decade has seen a succession of health alarms regarding 'foodborne diseases' and 'waterborne diseases', increasing attention to food and water safety (Pintó et al., 2009; EFSA, 2011; Sánchez and Bosch, 2016). However, data on viral contamination of food and water in Sicily are scarce and fragmentary. This is the first study to analyze both matrices for a panel of enteric viruses.

Among the wide range of enteric viruses, seven groups – HAV, NoV, EV, RoV, HEV, AdV, and BoV – were selected for this study due

Table 2
Detection of enteric viruses in shellfish, water, vegetables, food preparations, and swab samples.

	Number	Number and percentage of positive samples								
		HAV	GI NoV	GII NoV	GIV NoV	EV	RoV	HEV	AdV	BoV
Total	240	14	14	11	3	11	1	3	2	4
		5.8%	5.8%	4.6%	1.2%	4.6%	0.4%	1.2%	0.8%	1.7%
Shellfish	108	14	11	6	3	8	0	1	2	4
		13%	10.2%	5.6%	2.8%	7.4%	–	0.9%	1.9%	3.7%
Water	23	0	0	5	0	0	1	1	0	0
		–	–	21.7%	–	–	4.3%	4.3%	–	–
Vegetables	70	0	2	0	0	2	0	1	0	0
		–	2.9%	–	–	2.9%	–	1.4%	–	–
Food	11	0	0	0	0	1	0	0	0	0
		–	–	–	–	9%	–	–	–	–
Swabs	28	0	0	0	0	0	0	0	0	0
		–	–	–	–	–	–	–	–	–

HAV, hepatitis A virus; NoV, norovirus; EV, enterovirus; RoV, rotavirus; HEV, hepatitis E virus; AdV, adenovirus; BoV, bocavirus.

to their epidemiological significance as foodborne and waterborne pathogens (EFSA, 2011; La Rosa et al., 2012; Sánchez and Bosch, 2016). The study found an abundance of viruses in samples from shellfish, water, and vegetables and provided information on the presence of contamination in the environment, chiefly in shellfish production areas, which is useful for generating a picture of the circulation of viral pathogens able to infect humans.

Across all samples, HAV showed the highest percentage of presence (5.8%), followed by GI NoV (5.8%), EV (4.6%), and GII NoV (4.6%). Low percentages of BoV (1.7%), HEV (1.2%), GIV NoV (1.2%), AdV (0.8%), and RoV (0.4%) were found. In particular, high positivity for HAV (13%) was identified in bivalve mollusc shellfish samples, which were investigated as sentinel surveillance for marine pollution. No HAV was detected in the other matrices studied.

A recent outbreak of HAV in Korea had a reported attack rate of 14.6% (12/82); 11% (9/82) were symptomatic and one person died (Shin et al., 2017). Iaconelli et al. (2015) assessed the occurrence of HAV in shellfish samples and detected the virus in 23.2% of samples: 12 genotype IB and one genotype IA. La Rosa et al. (2014) investigated the spread of HAV in Italy through the monitoring of raw urban sewage and detected several variants, with prevalence of the IB strain having a countrywide distribution.

Of the 23 water samples in the present study, 21.7% were positive for GII NoV. Several studies have reported the detection of enteric viruses in water samples worldwide (La Rosa et al., 2010a,b; La Rosa et al., 2014; Kamel et al., 2011; Prado et al., 2012; Osuolale and Okoh, 2015), suggesting the contamination of aquatic environments. In a study of five municipal wastewater treatment plants located in central Italy, the concentration of AdV was highest in both raw and treated water compared with EV and NoV (GI and GII) (La Rosa et al., 2010b). During March 2011, an outbreak of gastroenteritis occurred in Agrigento, Sicily and NoV was identified in stool samples and in water samples from the public water system (Giammanco et al., 2014). In May 2016, a NoV gastroenteritis outbreak occurred at a seaside resort near Taormina (Mascali, Sicily), which originated from the municipal water distribution system (Giammanco et al., 2018).

In the present study NoV were also detected in shellfish (9.3% GI, 5.6% GII), and at a low percentage in vegetables (2.9%). NoV repeatedly cause outbreaks, either waterborne or associated with lettuce or shellfish probably contaminated with human faecal material used as fertilizer (Müller et al., 2016). However, the source is not identified in many outbreaks. In a meta-analysis of NoV outbreaks in nursing homes, foodborne introduction was described for 7%, and only 0.7% of outbreaks were reported to be foodborne, 28.5% person-to-person, and 70.8% remained unknown or not mentioned (Petrignani et al., 2015).

HBoV was detected in 3.7% of shellfish. Recently, the presence of HBoV in environmental samples has also been reported in Italy and worldwide (Iaconelli et al., 2016; La Rosa et al., 2017). La Rosa et al. (2018) detected HBoV in bivalve shellfish with a relevant prevalence, and reported the presence of HBoV in bivalve molluscs for the first time. In particular, the PCR products of three *Mytilus galloprovincialis* and one *Tapes decussates* were sequenced and characterized as HBoV-2 (three samples) and HBoV-3 (one sample previously detected positive for HAV IB).

The genetic heterogeneity of the viral strains from the positive Sicilian samples was particularly interesting, highlighting the presence of HAV IA and IB, of six different genotypes of GI NoV (GI.1, GI.2, GI.3, GI.5, GI.6, GI.8), and of three genotypes of GII NoV (GII.1, GII.2, GII.4) in the food matrices. A single genotype was detected for GIV NoV (GIV.1), HEV (G3), and AdV (AdV40). The present study detected the presence of HBoV in bivalve molluscs, confirming the results obtained in a previous investigation by our group (La Rosa et al., 2018).

This is the first surveillance study to show representative data from Sicily regarding food and environmental contamination by human enteric viruses. The information is essential for informed risk assessment of foodborne and waterborne diseases and the development of risk management decisions.

The study findings clearly demonstrate that human pathogenic viruses are widely found in aquatic environments and on vegetables. The results could be used as scientific support for defining appropriate strategies and methodologies to assess the public health risk linked to contamination and to implement regulations and systemic virological controls that can guarantee a high protection level applicable to the entire food supply chain. An integrated surveillance system including food safety and environmental and clinical human cases is clearly needed. It is important to obtain molecular data on virus isolated from humans and the environment. Such an integrated alert system would help rapidly understand possible future outbreaks and epidemics.

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Ethical approval

Ethical approval was not required.

Conflicts of interest

The authors report no conflicts of interest.

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